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Unvalidated antibodies and misleading results

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To the Editor

We would like to raise caution regarding the results of a recent publication in the July issue of Breast Cancer Research and Treatment by Muenst et al. This article entitled “Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer” appears to have arrived at an unreliable conclusion due to technical reasons. The investigators used an unvalidated polyclonal antibody to assess the expression of PD-L1. They have not provided validation to prove that the antibody binds to PD-L1. It is concerning that in Fig. 1, they show clear-cut, predominantly nuclear localization for a cytoplasmic and membrane protein. In a recent paper [10] we test four commercially available antibodies to PD-L1 that were all marketed as potentially suitable for immunohistochemistry but in our hands, only 1 validated using FFPE preparations from cell line PD-L1 transfectants and human term placenta as control for endogenous/native PD-L1 expression. We have expanded this effort in a recent ASCO poster showed only 2 of 7 PD-L1 antibodies validated when tested for specificity. In another publication, we show that both the protein and the mRNA are associated with better prognosis in two independent breast cancer cohorts [8]. We have also recently submitted an assessment of PD-L1 in a neo-adjuvant breast cancer cohort where high PD-L1 is associated with PathCR. The results by Muenst et al. are also contrary to numerous reports in the literature that show expression is associated with good prognosis in Melanoma [9]; colorectal carcinomas [5], and Merkel Cell carcinomas [6]; rather than the poor prognosis.

We believe that it is imperative to first rigorously validate any antibody for the purpose that it is used. Poorly validated antibodies may provide misleading information and contribute to

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the much larger issue of significant non-reproducibility of published research results [2, 7]. Our group has highlighted this problem in several publications [1], and we have described series of assays that can be easily done to show antibody sensitivity, specificity and reproducibility (validation) [3]. There are some published guidelines for IHC and FISH (called MISFISHIE [4]), they are not widely used. More recently the Global Biological Standards Institute (see www.gbsi.org) focused a task force on developing more rigorous standards for IHC and analytical reporting, but they have not yet produced a document.

Thus, the problem may be clearer than the solution. This letter is written in hopes of minimizing future erroneous publications by drawing attention to the necessity of in-house validation of reagents before reporting results.

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